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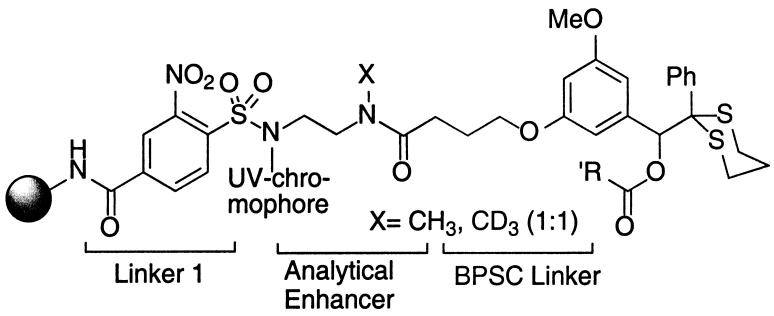

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Studies on the Chemical Stability and Functional Group Compatibility of the Benzoin Photolabile Safety-Catch Linker Using an Analytical Construct

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A chemical stability study of the benzoin photolabile safety-catch linker (BPSC) has been carried out using a dual-linker analytical construct to establish its compatibility with a range of commonly employed solid-phase reaction conditions. As a result of this study, the dithiane-protected benzoin linker was shown to be reactive only toward strong acids and fluoride nucleophile. Furthermore, a scan of diverse functional groups thought to be unstable toward the safety-catch removal conditions has also been carried out. These data should provide assistance in future utilization of the BPSC for syntheses.

Introduction

Over the past 30 years solid-phase organic synthesis has matured into a valuable chemical technology.[1] Linker molecules play a key role in any successful synthetic strategy on solid phase. Ideally, cleavage conditions should be compatible with the product released and should not introduce impurities that are difficult to remove. Photocleavable linkers are particularly useful in this respect, offering a mild, neutral, and broadly orthogonal method of cleavage without the need for exogenous cleavage reagents. Of the established photolabile chemical systems, we have focused our attention on the dithiane-protected benzoin system and demonstrated its utility as a safety-catch linker for solid-phase chemistry.[2] As a first proof of concept, the linker was assembled on resin in near-quantitative yield using Corey−Seebach[3,4] dithiane addition.

More recently,[5] a solution-phase approach to the second generation of benzoin photolabile safety-catch (BPSC) linkers (**1**) provides direct access to a versatile intermediate of high purity that can be loaded onto any resin of choice. The dithiane group that serves as a safety catch against premature photoreaction is removed by mild oxidizing agent prior to photolysis. Irradiation at 350 nm releases the free acids quantitatively with the concomitant formation of the inert 2-phenyl-5,7-dialkoxybenzofuran, which remains attached to the resin (**3**) (Scheme 1).

A further important criterion for the linker to fulfill is to be inert under a wide range of reaction conditions. Herein, we report a rapid reaction scan of the benzoin linker, together with a study of the compatibility of functional groups toward the dithiane cleavage conditions, by means of a dual-linker analytical construct.

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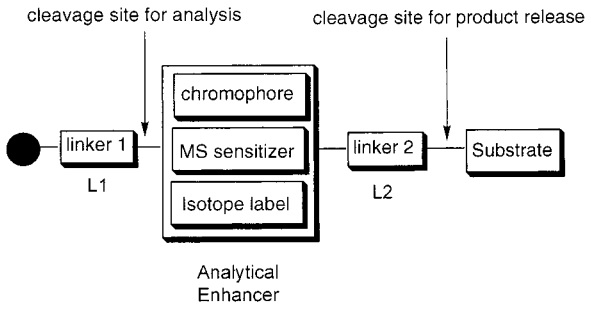

Figure 1. Schematic representation of the analytical construct. Cleavage at linker 2 (L2) releases the substrate. Alternatively, cleavage at linker 1 (L1) releases the substrate bound to an analytical unit (A) that enables characterization by HPLC−MS.

The dual-linker analytical construct approach comprises a linear sequence of two chemically orthogonal linkers separated by an analytical enhancer (Figure 1).[6] Cleavage at linker 2 (L2) releases only the substrate of interest in a conventional manner, whereas cleavage at linker 1 (L1) affords the substrate still attached to the analytical enhancer, which facilitates identification of that product by LC−MS. This principle was refined in a recently reported construct[7] where the design of the analytical unit was further developed. This last approach leads to release of an analytical fragment composed of not only an amine group (generated at cleavage) to enhance detection by electrospray mass spectroscopy (i.e., an MS sensitizer) and an isotope label to generate a characteristic split-peak pattern in the mass spectrum (an MS splitter) but also a UV chromophore for quick and accurate quantitation of products by HPLC−UV.

Results and Discussion

For this study, the dual-linker analytical construct **10** was used (Scheme 2). The *o*-nitrophenylsulfonamide group[8] was chosen as linker 1 and the dithiane-protected benzoin group derived from **1** as linker 2. On cleavage at linker 1, the MS

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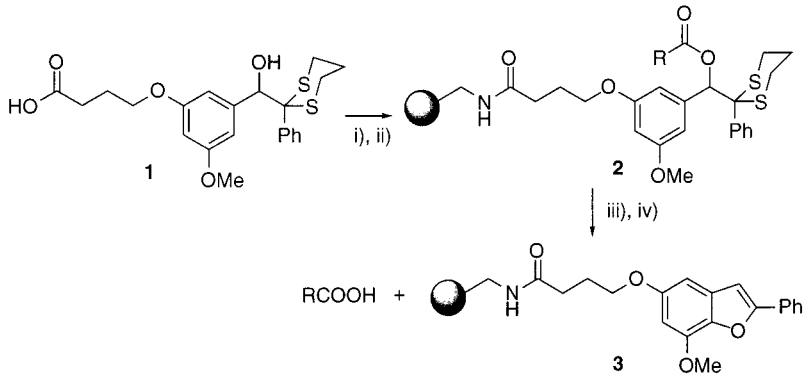
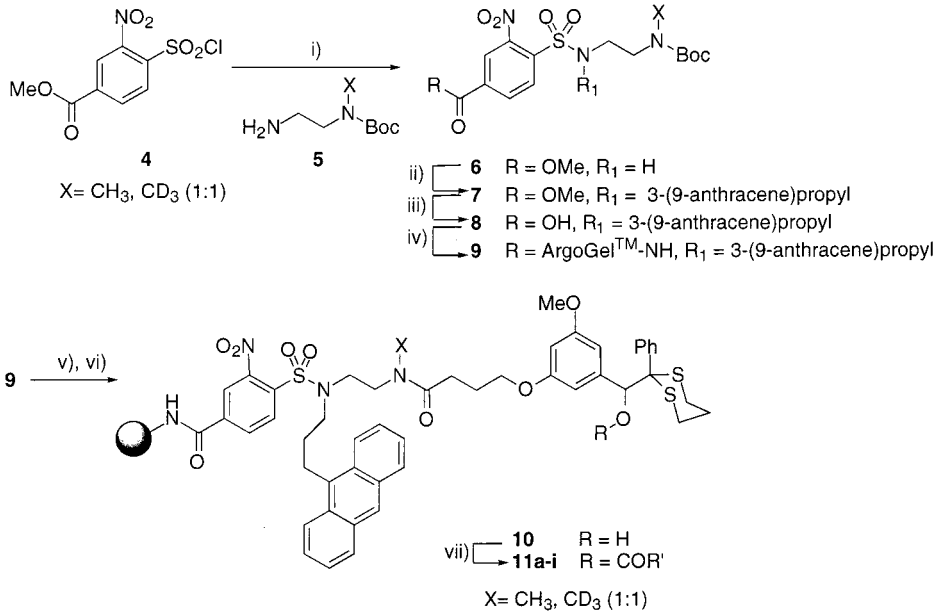
Scheme 1[a]



[a] (i) TentaGel amino resin (0.28 mmol/g, 1 equiv), TBTU (2.2 equiv), HOBt (2.5 equiv), $^{i}Pr_2NEt$ (4 equiv), compound **1** (2 equiv) in CH_2Cl_2/DMF (1:1), overnight. (ii) RCOOH (4 equiv), DIC (4 equiv), DMAP (0.5 equiv) in CH_2Cl_2, overnight. (iii) H_5IO_6 (2 equiv) in anhydrous THF, 30 min. (iv) Irradiation at 350 nm in THF/MeOH (3:1)

Scheme 2[a]



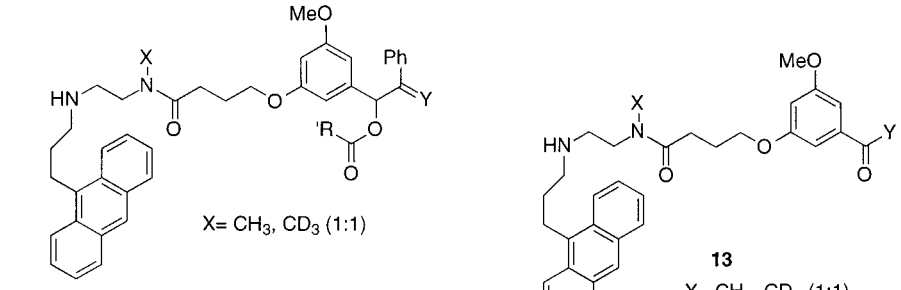
[a] (i) 4 (1 equiv), 5 (1 equiv), DIPEA (1.5 equiv), CH_2Cl_2, 0−20 °C, 17 h. (ii) Ps-BEMP (1.3 equiv), 9-(3-bromopropyl)anthracene (1.1 equiv), DMF, 20 °C, 14 h. (iii) NaOH (1.2 equiv), MeOH/THF (1:1), 3 h, 90%. (iv) ArgoGel-NH_2 resin (0.4 mmol g^{-1}, 0.75 equiv), DIC (2 equiv), HOBt (2 equiv), DMF, 20 °C, 16 h. (v) TFA/CH_2Cl_2 (1:1), 20 °C, 2 × 30 min. (vi) TBTU (2.2 equiv), HOBt (2.5 equiv), $^{i}Pr_2NEt$ (4 equiv), compound **1** (2 equiv) in CH_2Cl_2/DMF (1:1), overnight. (vii) R′COOH (4 equiv), DIC (4 equiv), DMAP (0.5 equiv) in CH_2Cl_2, overnight. **a**: R′ = CH_3. **b**: R′ = CH_2Ph. **c**: R′ = $CH_2CH{=}CH_2$. **d**: R′ = $CH_2CH_2C{\equiv}CH$. **e**: R′ = oxiranyl group. **f**: R′ = CH_2CH_2NH-Fmoc; **g** (derived from deprotection of Fmoc group of **11f**): R′ = $CH_2CH_2NH_2$. **h**: R′ = CH_2Br. **i**: R′ = *p*-(C_6H_4)-OTBS.

sensitizing amine tethered to the chromophore and the isotopic label bearing the BPSC linker is generated. Any chemical changes to the fully loaded BPSC unit, upon exposure to a range of conditions, can be readily monitored by this construct. The suitability of the 2-nitrophenyl sulfonamide linker for release of the amine component, in addition to its chemical compatibility, has been reported previously.[9] The anthracene moiety with a distinctive absorbance at 386 nm (ϵ_{386} = 9000, CH_3CN) has been demonstrated[7] to be a suitable probe to facilitate quantitation.

The preparation[10] of construct **10** is outlined in Scheme 2. The sulfonyl chloride **4** is reacted with the labeled *N*-[H_3,D_3]-methyl-*N*-*tert*-butoxycarbonylethylenediamine (**5**) to give sulfonamide **6**. N-Alkylation with 9-(3-bromopropyl)-anthracene, saponification of the methyl ester **7**, and subsequent coupling with ArgoGel TM amino resin (0.4 mmol g^{-1}) yield resin **9**. Following Boc deprotection of **9** with TFA, photolabile linker 1 was coupled using TBTU/HOBt in the presence of $^{i}Pr_2EtN$ to give the complete construct **10** in a purity of approximately 95%. The purity of resins **9** and **10** was assessed by cleavage at linker 1 using previously reported conditions[7] (thiophenol and $^{i}Pr_2EtN$ in acetonitrile) and analysis of the resulting filtrate by LC−MS.

The stability of the loaded benzoin linker toward the analytical cleavage conditions was studied initially. Construct **11a**, capped with acetic anhydride, was subjected to cleavage at the sulfonamide linker 1 with PhSH/ $^{i}Pr_2EtN$ in acetonitrile. Analysis by LC−MS of the resulting filtrate showed the corresponding analytical fragment **12a** (Figure 2) in a purity of approximately 95%, proving that both the ester linkage and the benzoin system are stable under these conditions.

The reaction scan on the benzoin linker was performed using the more robust benzyl ester derivative (construct **11b**). Incubation of bead aliquots to a range of commonly used



12a R' = CH_3, Y = $-S(CH_2)_3S-$ M = 751
12b R' = CH_2Ph, Y = $-S(CH_2)_3S-$ M = 827
12c R' = CH_2Ph, Y = $-S^+CH_3(CH_2)_3S-$ M = 827 + 15
12d R' = CH_2Ph, Y = $-SO(CH_2)_3S-$ M = 827 + 16
12e R' = CH_2Ph, Y = O M = 737

Figure 2. Analytical fragment released in the reaction scan study.

Table 1. Reaction Scan for the Benzoin Linker in Construct **11b**

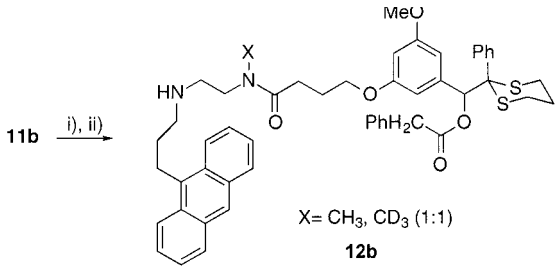
entry	reaction conditions[a]	≥85% of **12b**[b]	product obtained
	amide/ester coupling protocols		
1	DIC, HOBt, HOAc, DMAP, $^i Pr_2EtN$	√	
2	$^i Pr_2EtN$, DMAP cat., Ac_2O	√	
3	PhCOCl, pyr	√	
4	$PhCH_2Br$, $CsCO_3$	√	
5	$PhCH_2OH$, PPh_3, DEAD	√	
	alkylating/nucleophilic conditions		
6	NaH, $PhCH_2Br$	√	
7	MeMgBr (3 M)	√	
8	TBAF (1 M)	x	**13**
9	TBAF (1 M), MeI	x	**12c**
	carbon−carbon bond formation reactions		
10	$Pd(PPh_3)_4$, $PhB(OH)_2$, Na_2CO_3	√	
11	$Ph_3P{=}CH(CO)Me$	√	
	reductive conditions		
12	$BuNH_2$, $NaBH(OAc)_3$	√	
13	$NaBH_4$	√	
	oxidizing conditions		
14	H_2O_2 (10%)	√	
15	Pyr·SO_3, Et_3N	√	
16	NMO, Pr_4NRuO_4	x	**12d**
17	$NaIO_4$	x	**12d**
18	H_5IO_6, 30 min	x	**12e**
	bases		
19	20% piperidine	√	
20	$BuNH_2$, NMP	√	
	acids		
21	*p*-$TolSO_3H$	√	
22	$AcOH/H_2O/THF$ (3:1:1)	√	
23	BF_3·OEt_2	x	decomposition
24	TFA/DCM (1:1)	x	decomposition

[a] Resin aliquots, ∼20 mg, were incubated with reagents (5−10 equiv) in sealed 10 μL ShaftGuard FinePoint Rainin aerosol-resistant pipet tips (Anachem) at room temperature for 3 h and then washed and cleaved with PhSH/$^i Pr_2EtN$ in CH_3CN. LC−MS analyses were performed on a Hewlett-Packard HP 1050 instrument (diode array detection at 386 nm) and a Micromass Platform mass spectrometer using electrospray ionization in +ve mode. [b] √ indicates ≥85% **12b** by peak area. x indicates observed reactivity leading to the result quoted in column 4.

reagents[11] was performed in parallel, followed by subsequent washing of the resin, analytical cleavage, and analysis by LC−MS (Table 1, Scheme 3).

The results from this study reveal that the benzoin linker is stable to a wide range of reaction conditions including reductive, mild alkylating agents, basic and moderate acidic conditions, amide or ester coupling protocols, and Suzuki or Wittig reagents. Under the oxidizing conditions of entries 16 and 17, cleavage at linker 1 gave an analytical fragment we believe to be **12d** with a gain of 16 units of mass relative to the expected **12b**. Similarly, under reaction conditions of entry 9 the gain of 15 units of mass was shown after analytical cleavage. In these cases, the integrity of the analytical unit (A) itself (L1 + A, Figure 1) was further investigated. Thus, when resin **9** is submitted to reaction conditions of entries 9, 16, and 17 (Table 1), the analytical cleavage showed a fragment of 392 units of mass corresponding to *N*-methyl-*N*-Boc-*N*′-3-(9-anthracene)propylethylenediamine without any modification. Therefore, it can be concluded that it is the dithiane-protected benzoin linker that reacts with reactive alkylating agents such as methyl iodide, presumably by S-alkylation to give **12c** or with oxidizing reagents to give the corresponding sulfoxide **12d** (Figure 2). While this type of reactivity was not unexpected, the

Scheme 3[a]

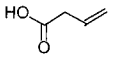
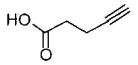
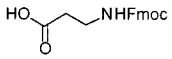
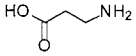
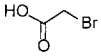
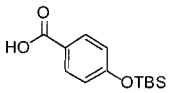


[a] (i) Reaction conditions. (ii) PhSH/DIPEA.

compatibility of the dithiane moiety toward milder oxidizing and alkylating agents is noteworthy (entries 6, 14, and 15). Under strong acidic conditons, such as $BF_3{\cdot}OEt_2$ or TFA (entries 23, 24, Table 1), decomposition of the BPSC linker is observed where loss of the acid moiety could be detected by LC−MS of the analytical fragment. Treatment of construct **11b** with a 1 M solution of TBAF promoted a retro-aldol type reaction in the benzoin linker, giving rise to the 3,5-dialkoxybenzaldehyde analytical fragment derivative partially oxidized to the acid under the phenyl thiolate cleavage (mixture **13**, Figure 2). This type of reactivity has been previously observed during the synthesis of linker 1 in solution.[5]

Another critical issue for the use of the BPSC linker concerns the stability of functionalities toward the periodic acid treatment necessary for safety-catch removal prior to photolysis. The oxidizing properties of periodic acid have been recognized since 1928, when Malaprade[12] first described its use in carbohydrate synthesis. Thereafter,[13] periodic acid has been used extensively for cleavage of 1,2-hydroxyaldehydes, -ketones, and -acids, 1,2-diketones, 1,2-aminoalcohols and -aldehydes, certain activated methylene groups, and cyclic 1,3-diketones. Non-Malapradian reactions (i.e., different from those due to overoxidation of carbohydrates) include[14] oxidation of condensed aromatic hydrocarbons, nonbenzenoid hydrocarbons, phenols, amines, derivatives of hydrazine, hydroxamic acids, indoles, and tryptophan derivatives. Despite the broad reactivity of H_5IO_6 toward a wide range of organic compounds, most of these reactions include the use of water and/or extended reaction times. The removal of the safety catch in the BPSC linker is efficient using only 2 equiv of H_5IO_6 in dry THF for less than half an hour. Therefore, using analytical construct **10**, we wished to confirm the chemoselectivity of this reagent toward the sulfur atoms of the dithiane group in the presence of functionalities prone to react with H_5IO_6, such as amines, alkenes, and epoxides. Initially the reaction conditions optimized for the removal of the dithiane safety catch were tried on construct **11b** (entry 18, Table 1), resulting in a clean and complete deprotection to the analytical fragment **12e** without oxidation of the anthracene moiety. Subsequently, construct **10** was loaded with the acids shown in Table 2. Periodic acid treatment of bead aliquots followed by the usual washing of resin and analytical cleavage showed a complete deprotection of the dithiane moiety. More importantly, all functionalities tested were found to be stable under these conditions except for the phenol derivative **11i** where the cleavage of the *tert*-butyldimethylsilyl (TBS) group and oxidation of the aromatic ring were the main side-reactions as observed by LC−MS analysis.

Table 2. Acids Loaded onto Construct **10** Bearing Different Functional Groups

Acid	Construct	Dithiane deprotection[a]	Functional group integrity[b]
	**11c**	√	√
	**11d**	√	√
	**11e**	√	√
	**11f**	√	√
	**11g**	√	√
	**11h**	√	√
	**11i**	√	x

[a] Resin aliquots, ∼5 mg, were incubated with H_5IO_6 (2 equiv) in sealed 10 μL ShaftGuard FinePoint Rainin aerosol-resistant pipet tips (Anachem catalog) at room temperature for 0.5 h and then washed and cleaved with PhSH/^{i}Pr$_2$EtN in CH_3CN. LC−MS analyses were performed on a Hewlett-Packard HP 1050 instrument (diode array detection at 386 nm) and a Micromass Platform mass spectrometer using electrospray ionization in +ve mode. [b] √ indicates ≥85% (by peak area) of the starting ester recovered intact after periodic acid treatment. x indicates that the ester derivative **11i** is not stable to the periodic acid treatment.

Conclusion

In conclusion, we have demonstrated that the benzoin photolabile safety-catch linker can be employed for a wide spectrum of reaction conditions. Besides the inherent reactivity of the safety catch toward oxidizing and alkylating agents, the dithiane-protected benzoin linker was found to be only reactive toward strong acids and fluoride nucleophile from the reaction conditions tested. Moreover, the mild reaction conditions used for the dithiane removal make it compatible with functional groups known to be reactive toward periodic acid. The analytical construct approach has proved to be valuable for providing a broad inventory of chemical compatibility for the BPSC linker. The information provided will facilitate synthetic planning and successful application of this linker for library synthesis in the future.

Acknowledgment. We acknowledge GlaxoSmithKline PLC for financial support.

Supporting Information Available. Experimental procedures for the preparation of compounds **6**−**8** and resins **10** and **11a**−**i**, general conditions for reaction scan on resin **12b** and for analytical cleavage, LC−MS traces for representative analytical cleavage of resins **10**, **11a**, and **11c**−**h**, compounds **12b**−**d**, and mixture **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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